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(54) Title: COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

(57) Abstract

A combination of a compound of the somatostatin class and a rapamycin macrolide is useful for the prevention or treatment of cell hyperproliferation.

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COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

The present invention relates to a pharmaceutical combination and its use in the treatment of disorders associated with excess benign and malignant cell proliferation, e.g. tumors or intimal cell proliferation.

There is a continuing need for the development of drugs having increased effectiveness in inhibiting or slowing down undesired cell proliferation, particularly in the cancer field and in vasculopathies.

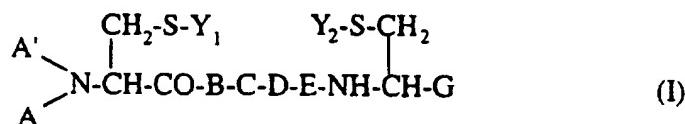
Accordingly, there is provided a pharmaceutical combination comprising a compound of the somatostatin class, and a rapamycin macrolide.

The somatostatin class is a known class of small peptides comprising the naturally occurring somatostatin-14 and analogues having somatostatin related activity, e.g. as disclosed by A.S. Dutta in Small Peptides, Vol.19, Elsevier (1993). By "somatostatin analogue" as used herein is meant any straight-chain or cyclic polypeptide having a structure based on that of the naturally occurring somatostatin-14 wherein one or more amino acid units have been omitted and/or replaced by one or more other amino radical(s) and/or wherein one or more functional groups have been replaced by one or more other functional groups and/or one or more groups have been replaced by one or several other isosteric groups. In general, the term covers all modified derivatives of the native somatostatin-14 which exhibit a somatostatin related activity, e.g. they bind to at least one somatostatin receptor (hSST-1, hSST-2, hSST-3, hSST-4 or hSST-5), preferably in the nMolar range, more preferably to at least the hSST-2 receptor in the nMolar range.

Cyclic, bridge cyclic and straight-chain somatostatin analogues or derivatives are known and have been described together with processes for their production e.g. in US Patent Specifications 4,310,518 and 4,235,886, in European Patent Specifications EP-A-1295; 23,192; 29,310; 29,579; 30,920; 31,303; 63,308; 70,021; 83,305; 215,171; 203,031; 214,872; 143,307; 298,732; 277,419; 389,180; 395,417; 450,480A2; in Belgian Patent Specification BE-A-900,089; and in WO 91/09056; WO 97/01579; WO 97/14715,

the contents thereof, in particular with respect to the compounds, being incorporated herein by reference.

Preferred somatostatin analogues are e. g. compounds of formula I



wherein

A is C₁₋₁₂alkyl, C₇₋₁₀phenylalkyl or a group of formula RCO-,

whereby

- i) R is hydrogen, C₁₋₁₁alkyl, phenyl or C₇₋₁₀phenylalkyl, or
- ii) RCO- is
 - a) a D-phenylalanine residue optionally ring-substituted by halogen, NO₂, NH₂, OH, C₁₋₃alkyl and/or C₁₋₃alkoxy; or
 - b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or
 - c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C₁₋₁₂alkylated or substituted by C₁₋₈alkanoyl;

A' is hydrogen or C₁₋₃alkyl,

Y₁ and Y₂ represent together a direct bond or each of Y₁ and Y₂ is hydrogen

B is -Phe- optionally ring-substituted by halogen, NO₂, NH₂, OH, C₁₋₃alkyl and /or

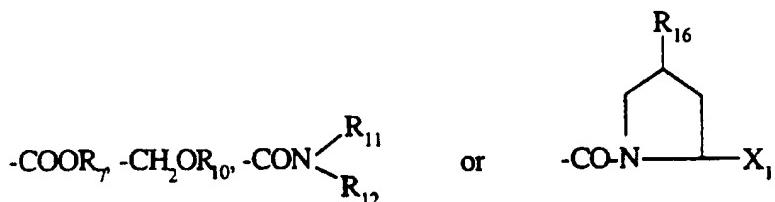
C_{1-3} alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α -N-methylated and optionally benzene-ring-substituted by halogen, NO_2 , NH_2 , OH, C_{1-3} alkyl and/or C_{1-3} alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue

G is a group of formula



wherein

R_7 is hydrogen or C_{1-3} alkyl,

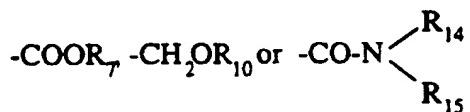
R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester, e.g. formyl, C_{2-12} alkylcarbonyl, benzoyl,

R_{11} is hydrogen, C_{1-3} alkyl, phenyl or C_{7-10} phenyl-alkyl,

R_{12} is hydrogen, C_{1-3} alkyl or a group of formula $-CH(R_{13})-X_1$,

R_{13} is CH_2OH , $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



wherein

R₇ and R₁₀ have the meanings given above,

R₁₄ is hydrogen or C₁₋₃alkyl and

R₁₅ is hydrogen, C₁₋₃alkyl, phenyl or C₇₋₁₀phenylalkyl, and

R₁₆ is hydrogen or hydroxy,

with the proviso that

when R₁₂ is -CH(R₁₃)-X, then R₁₁ is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration,

in free form or in pharmaceutically acceptable salt or complex form.

Individual compounds of formula I suitable in accordance with the present invention are the following somatostatin analogues:

a. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

also known as octreotide

b. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH₂

c. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH₂

also known as vapreotide

d. (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH₂

e. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH₂

f. 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH₂

also known as lanreotide

- g. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys- β -Nal-NH₂
- h. 3-(2-naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys- β -Nal-NH₂
- i. (D)Phe-Cys- β -Nal-(D)Trp-Lys-Val-Cys-Thr-NH₂
- j. (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH₂
- k. (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH₂.

A preferred compound of formula I is octreotide.

Compounds of formula I may exist e.g. in free form, salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and acetates. Complexes are e.g. formed from compounds of the invention on addition of inorganic substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or an addition of polymeric organic substances.

Further somatostatin analogues suitable for use in accordance with the present invention are:

cyclo [-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Gaba-],

cyclo(Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser), and

(D)Nal-Glu-Tyr-(D)Trp-Lys-Val-Lys-Thr-NH₂

According to an alternatively preferred embodiment of the invention, the somatostatin component of the combination is a somatostatin analogue comprising the amino acid sequence of formula (II)



wherein X_2 is a radical of formula (a) or (b)



or



wherein R_1 is optionally substituted phenyl,

R_2 is $-Z_1\text{-CH}_2\text{-R}_1$, $-\text{CH}_2\text{-CO-O-CH}_2\text{-R}_1$,



wherein Z_1 is O or S,

and

X_3 is an α -amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(BzI)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys⁹ of the native somatostatin-14.

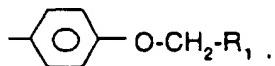
Such somatostatin analogues are e.g. disclosed in WO/ 97/01579, the contents thereof, in particular with respect to the specifically exemplified compounds, being

incorporated herein by reference.

Preferably the sequence of formula II as defined above corresponds to the residues at positions 8 through 11 of the somatostatin-14. More preferably the somatostatin analogue as disclosed above comprises a hexapeptide unit, the residues at positions 3 through 6 of said hexapeptide unit comprising the sequence of formula II. More particularly the hexapeptide unit is cyclic, e.g. having a direct peptide linkage between the α -carbonyl group of the residue at position 6 and the α -amino group of the residue at position 1.

While Lys, X_2 and X_3 in the sequence of formula II have the L-configuration, Trp may have the D- or L-configuration, preferably the D-configuration.

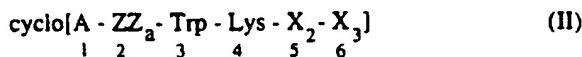
X_2 is preferably a residue of formula (a) or (b), R_2 being preferably $-Z_1\text{-CH}_2\text{-R}_1$, or



When X_3 comprises an aromatic residue on the C_α side chain, it may suitably be a natural or unnatural α -amino acid, e.g. Phe, Tyr, Trp, Nal, Pal, benzothienyl-Ala, Tic and thyronin, preferably Phe or Nal, more preferably Phe. X_3 is preferably an α -amino acid bearing an aromatic residue on the C_α side chain.

When R_1 is substituted phenyl, it may suitably be substituted by halogen, methyl, ethyl, methoxy or ethoxy e.g. in ortho and/or para. More preferably R_1 is unsubstituted phenyl. Z_1 is preferably O.

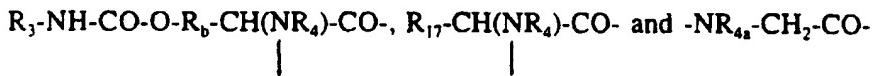
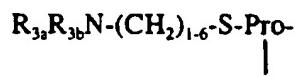
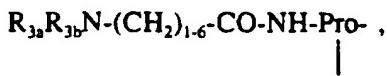
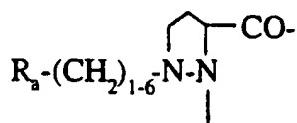
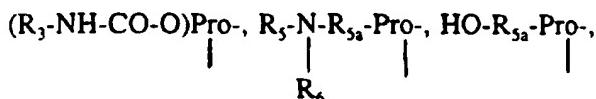
Representative somatostatin analogues comprising a residue of formula II are e.g. compounds of formula (III)



wherein

X_2 and X_3 are as defined above,

A_1 is a divalent residue selected from Pro,



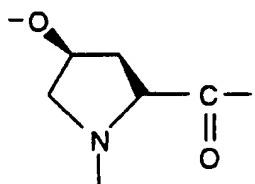
wherein R_3 is $NR_8R_9-C_{2-6}\text{alkylene}$, guanidino- $C_{2-6}\text{alkylene}$ or $C_{2-6}\text{alkylene-COOH}$, R_{3a} is H, $C_{1-4}\text{alkyl}$ or has independently one of the significances given for R_3 , R_{3b} is H or $C_{1-4}\text{alkyl}$, R_8 is OH or NR_5R_6 , R_b is $-(CH_2)_{1-3}-$ or $-CH(CH_3)-$, R_4 is H or CH_3 , R_{4a} is optionally ring-substituted benzyl, each of R_5 and R_6 independently is H, $C_{1-4}\text{alkyl}$, $\omega\text{-amino-}C_{1-4}\text{alkylene}$, $\omega\text{-hydroxy-}C_{1-4}\text{alkylene}$ or acyl, R_{5a} is a direct bond or $C_{1-6}\text{alkylene}$, each of R_8 and R_9 independently is H, $C_{1-4}\text{alkyl}$, $\omega\text{-hydroxy-}C_{2-4}\text{alkylene}$, acyl or $CH_2OH-(CHOH)_c-CH_2-$ wherein c is 0, 1, 2, 3 or 4, or R_8 and R_9 form together with the nitrogen atom to which they are attached a heterocyclic group which may comprise a further heteroatom, and R_{17} is optionally ring-substituted benzyl, $-(CH_2)_{1-3}-OH$, $CH_3-CH(OH)-$ or $-(CH_2)_{1-5}-NR_5R_6$, and

ZZ_1 is a natural or unnatural α -amino acid unit.

ZZ_1 may have the D- or L-configuration. When ZZ_1 is a natural or unnatural α -amino acid unit, it may suitably be e.g. Thr, Ser, Ala, Val, Ile, Leu, Nle, His, Arg, Lys, Nal, Pal,

Tyr, Trp, optionally ring-substituted Phe or N^a-benzyl-Gly. When ZZ₁ is Phe, the benzene ring thereof may be substituted by e.g. NH₂, NO₂, CH₃, OCH₃, or halogen, preferably in para position. When ZZ₁ is Phe, the benzene ring thereof is preferably unsubstituted.

When A₁ comprises a Pro amino acid residue, any substituent present on the proline ring, e.g. R₃-NH-CO-O- etc., is preferably in position 4. Such substituted proline residue may exist in the cis form, e.g.



as well as in the trans form. The present invention covers each geometric isomer individually as well as mixtures thereof.

When A₁ is (NR₈R₉-C₂₋₆alkylene-NH-CO-O)Pro- where NR₈R₉ forms a heterocyclic group, such group may be aromatic or saturated and may comprise one nitrogen or one nitrogen and a second heteroatom selected from nitrogen and oxygen. Preferably the heterocyclic group is e.g. pyridyl or morpholino. C₂₋₆Alkylene in this residue is preferably -CH₂-CH₂-.

Any acyl as R₅, R₆, R₈ and R₉ in A₁ may be e.g. R₁₈CO- wherein R₁₈ is H, C₁₋₄alkyl, C₂₋₄alkenyl, C₃₋₆cycloalkyl or benzyl, preferably methyl or ethyl. When R_{4a} or R₁₇ in A₁ is ring-substituted benzyl, the benzene ring may be substituted as indicated above for ZZ₁.

A preferred group of compounds of formula III are such wherein A₁ is free of a lateral -NH-CO-O- moiety. A further group of preferred compounds of formula III are such wherein A₁ comprises a basic lateral radical, e.g. a R₃-NH-CO-O- or R₅-N-R_{5a}-



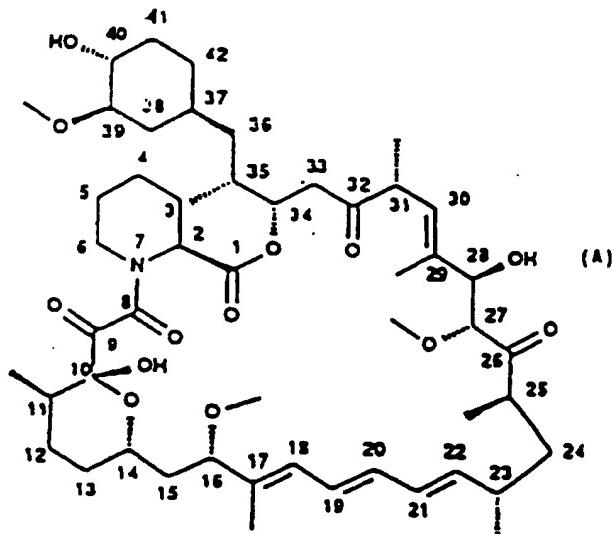
moiety.

A still further group of preferred compounds of formula III are such wherein the N-terminal amino acid comprises a substituted Pro, particularly 4-substituted Pro, e.g. compounds of formula III wherein A₁ is 4-substituted Pro.

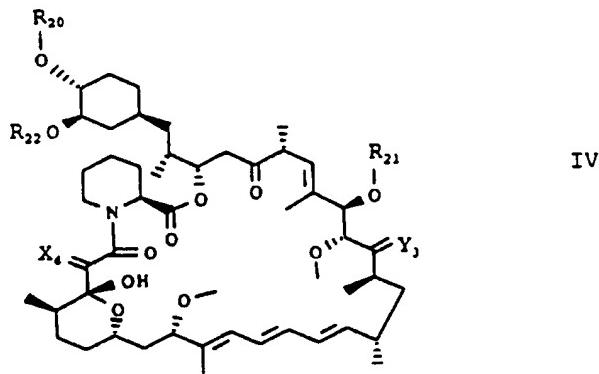
Preferably A₁ is 4-(R₃-NH-CO-O)Pro.

Examples of somatostatin analogues comprising a residue of formula II include e.g. cyclo[4-(NH₂-C₂H₄-NH-CO-O-)Pro-Phe-DTrp-Lys-Ser(Benzyl)-Phe].

The term "macrolide" as used herein, refers to a macrocyclic lactone, for example a compound having a 12-membered or larger lactone ring. Of particular interest are the "lactam macrolides", i.e. macrocyclic compounds having a lactam (amide) bond in the macrocycle in addition to a lactone (ester) bond, for example rapamycin and its numerous derivatives and analogues. Rapamycin is an immunosuppressive lactam macrolide that is produced by Streptomyces hygroscopicus, and having the structure depicted in Formula A:



See, e.g., McAlpine, J.B., et al., *J. Antibiotics* (1991) **44**: 688; Schreiber, S.L., et al., *J. Am. Chem. Soc.* (1991) **113**: 7433; US Patent No. 3 929 992. One group of rapamycin derivatives are 40-O-substituted derivatives of rapamycin having the structure of Formula IV:



wherein

X_4 is (H,H) or O;

Y_3 is (H,OH) or O;

R_{20} and R_{21} are independently selected from H, alkyl, arylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkoxycarbonylalkyl, hydroxyalkylarylalkyl, dihydroxyalkylarylalkyl, acyloxyalkyl, aminoalkyl, alkylaminoalkyl, alkoxycarbonylaminoalkyl, acylaminoalkyl, arylsulfonamidoalkyl, allyl, dihydroxyalkylallyl, dioxolanylallyl, dialkyl-dioxolanylalkyl, di(alkoxycarbonyl)-triazolyl-alkyl and hydroxyalkoxy-alkyl; wherein "alk-" or "alkyl" refers to C₁₋₆alkyl, branched or linear, preferably C₁₋₃alkyl; "aryl" is phenyl or tolyl; and acyl is a radical derived from a carboxylic acid; and

R_{22} is methyl or R_{22} and R_{20} together form C₂₋₆alkyl;

provided that R₂₀ and R₂₁ are not both H; and hydroxyalkoxyalkyl is other than hydroxyalkoxymethyl.

Such compounds are disclosed in WO 94/09010 the contents of which, in particular with respect to the specifically exemplified compounds, are incorporated herein by reference.

A preferred compound is e.g. 40-O-(2-hydroxy)ethyl-rapamycin (referred thereafter as Compound B).

Further preferred rapamycin derivatives are e.g. those disclosed in WO 96/41807, the contents thereof, in particular with respect to the specifically exemplified compounds of formula I disclosed therein, being incorporated herein by reference. Particularly preferred are 32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-40-O-(2-hydroxyethyl)-rapamycin, 16-O-pent-2-ynyl-32-(S)-dihydro-rapamycin and 16-O-pent-2-ynyl-32-(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin.

Further rapamycin derivatives are known, e.g. carboxylic acid esters such as disclosed in WO 92/05179, amide esters such as disclosed in US 5 118 677, carbamates such as described in US 5 118 678, fluorinated esters such as disclosed in US 5 100 883, acetals, e.g. in US 5 151 413, silyl ethers, e.g. in US 5 120 842, arylsulfonates and sulfamates, e.g. in US 5 177 203, derivatives wherein the methoxy group at the position 16 is replaced with alkynyoxy, e.g. in WO 95/16691 and further derivatives such as disclosed in WO 93/11130, WO 94/02136, WO 94/02385 and WO 95/14023, all incorporated herein by reference.

Rapamycin and above mentioned derivatives have been shown to have potent immunosuppressant properties. Rapamycin has also been shown to inhibit smooth muscle cell proliferation and to inhibit cancer growth.

Somatostatin analogues, e.g. octreotide, vapreotide and lanreotide, have been disclosed i.a. to inhibit growth hormone secretion and to have an inhibiting effect on malignant tumor growth, e.g. in breast cancer. Octreotide and lanreotide have also been disclosed to inhibit smooth muscle cell proliferation.

In accordance with the invention, it has now surprisingly been found that a combination of 2 active ingredients believed to act on basically different mechanisms such as a somatostatin analogue and rapamycin or a derivative thereof, can be combined and synergistically inhibit cell hyperproliferation.

In accordance with the particular findings of the present invention, there is provided in a first aspect:

1. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, for manufacturing a pharmaceutical composition for use in synergistically effective amounts in the prevention or treatment of cell hyperproliferation in combination with a rapamycin macrolide, e.g. for the manufacture of a kit as disclosed hereinafter.
2. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, in combination in synergistically effective amounts with a rapamycin macrolide for the prevention or treatment of cell hyperproliferation.
3. A method for preventing or treating cell hyperproliferation in a subject in need of such treatment which comprises administering to such subject a synergistically effective amount of a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a rapamycin macrolide.
4. A kit or package for the treatment or prevention of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a

pharmaceutical composition comprising a rapamycin macrolide. The kit or package may also contain instructions to use the pharmaceutical compositions in accordance with the present invention.

According to the invention, the combination of a compound of the somatostatin class and a rapamycin macrolide is indicated for the prevention or treatment of malignant tumor growth, e.g. breast, lung, GEP tumors, pituitary adenomas, lymphomas, etc., for the prevention or treatment of proliferative vascular diseases, e.g. biologically or mechanically induced vascular injury causing intimal thickening, e.g. restenosis, atherosclerosis, vascular occlusion, injury following percutaneous transluminal coronary angioplasty, vascular surgery or transplantation surgery, transplant vasculopathies, for example chronic rejection of various tissues and organs such as heart, kidney, pancreas, lung, liver, bowel, trachea and combined heart-lung.

The combination is particularly indicated for preventing intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion in a mammal.

Utility of the combination in the treatment of disorders and diseases as hereinbefore specified, may be demonstrated for example in accordance with the method hereinafter described.

A. In vitro Assay

AR42J cell cultures are propagated in DMEM supplemented with 10 % fetal calf serum (FCS) at 5 % CO₂. Cells are grown in the absence of antibiotics or antifungal agents. Subconfluent AR42J cells growing in DMEM and supplemented with 10 % FCS are trypsinized, diluted in DMEM + 2.5 % FCS and seeded in uncoated 96-well plates (5'000 to 10'000 cells per well in 180 µl). After a 48-hr incubation period (Day 0), the number of cells in a separate control plate is determined both by counting cells in a Coulter counter and by the sulforhodamine B (SRB) staining assay. The cells are then exposed either to the somatostatin analogue alone, e.g. octreotide, or to rapamycin or a derivative thereof alone or to a combination of the somatostatin analogue and rapamycin

or its derivative up to 5 days at various concentrations. Total drug exposure lasts for up to 5 days following the first addition and SRB analysis as described above is performed e.g. on day 2 and day 5. Growth is determined as difference in absorbance (OD) between day 0 and day x values (= delta OD). Calculations are made based on the fractional product method of Webb (Valeriote and Lin, 1975; Cory and Carter, 1986; Berenbaum, J. Theor. Biol. 114: 413-431, 1985) and the method by Chou and Talalay (Adv. Enz. Regul. 22: 27-55, 1984). If the measured cell growth (% of control) is < to the calculated cell growth, this shows evidence for a synergistic effect. Under these conditions a combination of a somatostatin analogue at a concentration of from 10^{-10} to 10^{-6} M with a rapamycin macrolide thereof at a concentration of from 1 to 1000 nM significantly inhibits the growth of the tumor cells.

In this assay, the following results are obtained with octreotide alone, Compound B alone and a combination of octreotide and Compound B. The synergy according to the Webb Method is confirmed by using the Chou-Talalay Method.

	Cell Growth (% of CONTROL)			
	Concentration (nM)	Cell Growth (Δ OD) (%)	Observed (%)	Calculated (Webb Method) (%)
Control		664 ± 9	100	
Octreotide	1.2	397 ± 16	59.8	
Compound B	12.0	420 ± 12	63.3	
Octreotide + Compound B	1.2 + 12.0	103 ± 5	15.6	37.9

B. In Vivo Assay

The AR42J (AR4-2J) rat pancreatic tumor cell line is derived from an azaserine-induced exocrine pancreatic tumor (Jessop and Hay, 1980). It was obtained from ATCC.

Cultures are propagated in DMEM supplemented with 10% fetal calf serum (FCS) at 5% CO₂. Cells are grown in the absence of antibiotics or antifungal agents. Female nude mice (nu/nu Balbc-A from Iffa Credo, Lyon, France) weighing 19-22 g, are kept in groups of 5 animals in macrolon cages (type III, 16 x 22 x 11 cm). The cages are placed in ventilated cabinets (Iffa Credo) that are maintained at 24 ± 1° C. The animals have free access to drinking water and a pathogen-free rodent diet (Diet A, Kliba, Basel, Switzerland). To initiate tumors from cultured cells, AR42J cells are trypsinized and 10x10⁶ tumor cells (in 0.2 ml) are injected subcutaneously (s.c.) into both flanks of nude mice. When tumors have reached a volume of 0.03 cm³, animals are randomized into control and treatment groups. Control animals receive placebo. Animals are treated as indicated below for 3 weeks with single agents or the drug combination. The somatostatin analogue is given as a single injection of a slow release form at 30 mg/kg s.c.. The size of the tumors is determined with a caliper. To calculate the tumor volume in ml the equation "volume (ellipsoid) = length x depth x height x 0.52" was used.

Results

After 4 weeks the following tumor size were determined.

(Please note that values in the control group correspond to 3 week values, since animals were killed afterwards for tumors became excessively large.)

Treatment	Volume mm ³	SE
Control	4020	579
A) Compound B, 5 mg/kg p.o.	3685	263
B) Rapamycin, 5 mg/kg p.o.	2748	325
C) Octreotide pamoate (biodegradable, sustained release formulation), 30 mg/kg, single inj.	2205	339

Compound B + octreotide (C)	130	75
Rapamycin + octreotide (C)	106	44

C. Clinical trial

Patients are included who have breast cancer as evidenced by histological biopsy (glandular analysis - EOA). They present a metastatic illness and/or loco-regional localisation which is measurable and evaluable. If desired, patients may be included who are resistant to other treatment to conventional therapy such as surgery, radiotherapy, other chemotherapy and/or hormone therapy.

The patients present at least one target, on X-ray analysis, which is measurable or evaluable such as a primitive metastatic tumour which is cutaneous or sub-cutaneous. It may be gangliar or visceral. Preferably the patients have lesions which have progressed within the month preceding the trial and have an estimated survival time of at least 3 months.

The rapamycin macrolide, e.g. rapamycin or compound B is administered orally. The treatment is for at least 3 months or until complete remission. The response may be followed by conventional methodology, e.g. according to IUCC response criteria, e.g. progression, stabilization, partial or complete remission.

The somatostatin analogue, e.g. octreotide, is administered parenterally, e.g. subcutaneous, particularly in a continuous subcutaneous way by means of a portable syringe pump (infusion pump).

According to the invention, the somatostatin analogue and the rapamycin macrolide are preferably administered in the form of a pharmaceutical composition. Rapamycin and its derivatives, e.g. Compound B, may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, emulsions or microemulsion preconcentrates, nasally, pulmonary (by inhalation),

parenterally, e.g. in the form of injectable solutions or suspensions, or topically. Rapamycin and its derivatives are preferably administered per os and the somatostatin analogue is preferably administered parenterally, e.g by infusion. The somatostatin analogue may also be administered in a slow release form, e.g. as disclosed in UK Patent Specification 2,265,311B. The administration of each component of the combination may take place either separately, simultaneously or sequentially, e.g. rapamycin or Compound B may be administered at first followed later, e.g. 8 to 24 hours later, by the somatostatin analogue.

The amount of each component administered is determined taking into account various factors such as the etiology and severity of the disease, and the patient's condition.

Rapamycin or its derivatives may conveniently be administered at doses which are in the range used in immunosuppressive applications such as prevention and treatment of graft vs. host disease, transplant rejection or autoimmune diseases e.g. at a daily dosage from about 0.5 to 500 mg as a single dose or in divided doses. Such doses may also be given intermittently, for example, every other day or every third day. The somatostatin analogue may be administered, e.g. subcutaneously, in a dosage range of about 100 µg to 10 mg per day as a single dose or in divided doses. Thus octreotide may be administered at a dose of from 0.2 mg to 10 mg twice or three times daily. When administered as a slow release form, such formulation may comprise the somatostatin peptide in a concentration from 2.0 to 10% by weight. The release period of such a formulation may be from 1 week to about 2 months. The combination of the somatostatin analogue with rapamycin or its derivative allows to maximize the antiproliferative effect.

The invention contemplates that the active ingredients discussed herein may be utilized in combination with pharmaceutically acceptable diluents and carriers.

Formulation Examples:A. Somatostatin Formulations:1. Ampoules

Octreotide	0.5 mg
Mannitol	45.0 mg
Lactic acid (88%)	3.4 mg
Sodium hydrogeno-	
carbonate	to pH 4.2
Water (inject.grade)	to 1 ml
Carbon dioxide	q.s.

2. Biodegradable sustained release formulation:

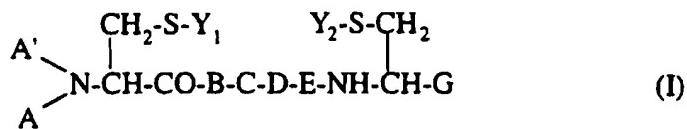
Octreotide Acetate	4.65 %	(by weight)
Poly(DL-lactide-co-glycolide)	78.35 %	
Sterile Mannitol	17 %	
Vehicle: Carboxymethylcellulose	0.5 %	(by weight)
Mannitol	0.6 %	
Water for injection	98.9 %	

B. Rapamycin (or derivative thereof) formulation: e.g. capsules

Ethanol	20.0 mg
1,2-propylene glycol	81.0mg
Refined oil	121.5mg
Cremophor RH40	202.5mg
Rapamycin or Compound B	20.0mg
Total	500 mg

CLAIMS

1. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, for manufacturing a pharmaceutical composition for use in synergistically effective amounts in the prevention or treatment of cell hyperproliferation in combination with a rapamycin macrolide.
2. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, in combination in synergistically effective amounts with a rapamycin macrolide for the prevention or treatment of cell hyperproliferation.
3. Use according to claim 1 or 2, wherein the compound of the somatostatin class is a compound of formula I



wherein

A is C_{1-12} alkyl, C_{7-10} phenylalkyl or a group of formula RCO- ,

whereby

- i) R is hydrogen, C_{1-11} alkyl, phenyl or C_{7-10} phenylalkyl, or
- ii) RCO- is
 - a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2 , NH_2 , OH , C_{1-3} alkyl and/or C_{1-3} alkoxy; or

- b) the residue of a natural or a synthetic α -amino-acid other than defined under a) above, or of a corresponding D-amino acid, or
- c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α -amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C₁₋₁₂alkylated or substituted by C₁₋₈alkanoyl;

A' is hydrogen or C₁₋₃alkyl,

Y₁ and Y₂ represent together a direct bond or each of Y₁ and Y₂ is hydrogen

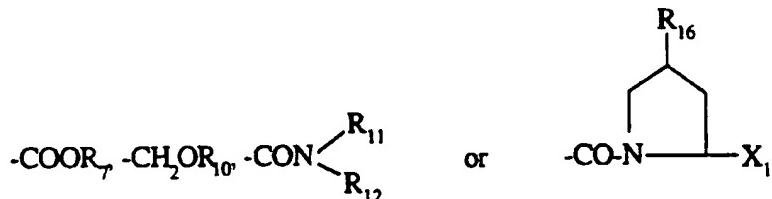
B is -Phe- optionally ring-substituted by halogen, NO₂, NH₂, OH, C₁₋₃alkyl and /or C₁₋₃alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α -N-methylated and optionally benzene-ring-substituted by halogen, NO₂, NH₂, OH, C₁₋₃alkyl and/or C₁₋₃alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue

G is a group of formula



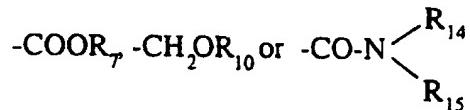
wherein

R₇ is hydrogen or C₁₋₃alkyl,

R₁₀ is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester,

R_{11} is hydrogen, C_{1-3} alkyl, phenyl or C_{7-10} phenyl-alkyl,
 R_{12} is hydrogen, C_{1-3} alkyl or a group of formula $-CH(R_{13})-X_1$,
 R_{13} is CH_2OH , $-(CH_2)_2-OH$, $-(CH_2)_3-OH$, $-CH(CH_3)OH$, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



wherein

R_7 and R_{10} have the meanings given above,
 R_{14} is hydrogen or C_{1-3} alkyl and
 R_{15} is hydrogen, C_{1-3} alkyl, phenyl or C_{7-10} phenylalkyl, and
 R_{16} is hydrogen or hydroxy,

with the proviso that

when R_{12} is $-CH(R_{13})-X_1$ then R_{11} is hydrogen or methyl.

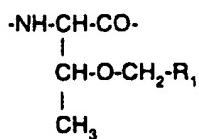
wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration

or a somatostatin analogue comprising the amino acid sequence of formula II

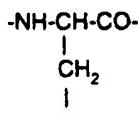


wherein X_2 is a radical of formula (a) or (b)

or



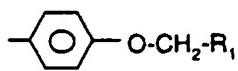
(a)



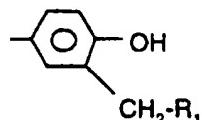
(b)

wherein R_1 is optionally substituted phenyl,

R_2 is $-\text{Z}_1\text{-CH}_2\text{-R}_1$, $-\text{CH}_2\text{-CO-O-CH}_2\text{-R}_1$,



or



wherein Z_1 is O or S,

and

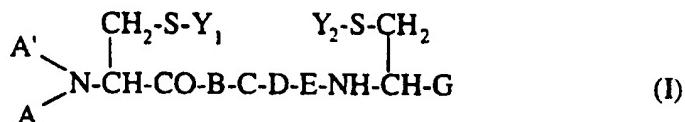
X_3 is an α -amino acid having an aromatic residue on the C_{α} side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys⁹ of the native somatostatin-14,

in free form or in pharmaceutically acceptable salt form.

4. Use according to claim 3, wherein the compound of the somatostatin class is octreotide, lanreotide or vapreotide.
5. A method for preventing or treating cell hyperproliferation in a subject in need of such treatment which comprises administering to such subject a synergistically effective amount of a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a rapamycin macrolide.

6. A kit or package for the treatment or prevention of cell hyperproliferation, said kit or package including a pharmaceutical composition comprising a compound of the somatostatin class in free form or in pharmaceutically acceptable salt form, and a pharmaceutical composition comprising a rapamycin macrolide, together with instructions for use.
7. A kit or package according to claim 6, wherein the compound of the somatostatin class is a compound of formula I



wherein

A is C₁₋₁₂alkyl, C₇₋₁₀phenylalkyl or a group of formula RCO-,
whereby

- i) R is hydrogen, C₁₋₁₁alkyl, phenyl or C₇₋₁₀phenylalkyl, or
- ii) RCO- is
 - a) a D-phenylalanine residue optionally ring-substituted by halogen, NO₂, NH₂, OH, C₁₋₃alkyl and/or C₁₋₃alkoxy; or
 - b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or
 - c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C₁₋₁₂alkylated or substituted by C₁₋₈alkanoyl;

A' is hydrogen or C₁₋₃alkyl,

Y₁ and Y₂ represent together a direct bond or each of Y₁ and Y₂ is hydrogen

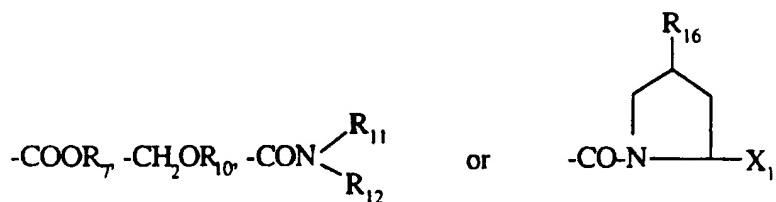
B is -Phe- optionally ring-substituted by halogen, NO₂, NH₂, OH, C₁₋₃alkyl and /or C₁₋₃alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO₂, NH₂, OH, C₁₋₃alkyl and/or C₁₋₃alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly

E is Thr, Ser, Val, Tyr, Ile, Leu or an aminobutyric or aminoisobutyric acid residue

G is a group of formula



wherein

R₇ is hydrogen or C₁₋₃alkyl,

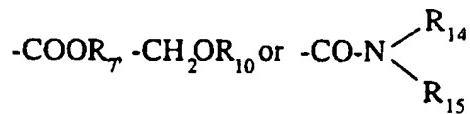
R₁₀ is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester,

R₁₁ is hydrogen, C₁₋₃alkyl, phenyl or C₇₋₁₀phenyl-alkyl,

R₁₂ is hydrogen, C₁₋₃alkyl or a group of formula -CH(R₁₃)-X₁,

R₁₃ is CH₂OH, -(CH₂)₂-OH, -(CH₂)₃-OH, -CH(CH₃)OH, isobutyl, butyl, benzyl, naphtyl-methyl or indol-3-yl-methyl, and

X₁ is a group of formula



wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3} alkyl and

R_{15} is hydrogen, C_{1-3} alkyl, phenyl or C_{7-10} phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

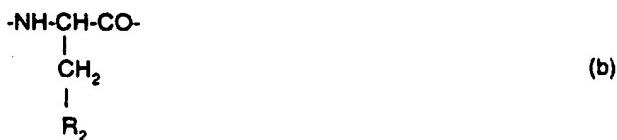
when R_{12} is $-\text{CH}(R_{13})-\text{X}_1$ then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)- configuration

or a somatostatin analogue comprising the amino acid sequence of formula II



wherein X_2 is a radical of formula (a) or (b)



or

wherein R₁ is optionally substituted phenyl,

R₂ is -Z₁-CH₂-R₁, -CH₂-CO-O-CH₂-R₁,



wherein Z₁ is O or S,

and

X₃ is an α -amino acid having an aromatic residue on the C_a side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

the residue Lys of said sequence corresponding to the residue Lys⁹ of the native somatostatin-14,

in free form or in pharmaceutically acceptable salt form.

8. A kit or package according to claim 7, wherein the compound of the somatostatin class is octreotide, lanreotide or vapreotide.
9. A kit or package according to claim 6 for simultaneous, separate or sequential use in synergistically effective amounts.

INTERNATIONAL SEARCH REPORT

Inte
nal Application No
PCT/EP 97/03036

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 239 178 A (SANDOZ LTD) 26 June 1991 see the whole document ---	1-9
A	WO 93 11130 A (SMITHKLINE BEECHAM PLC) 10 June 1993 cited in the application see the whole document ---	1-9
A	SHI E.A.: "Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro" CANCER RESEARCH, vol. 55, 1 May 1995, pages 1982-1988, XP002040888 see the whole document -----	1-9

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *'A' document defining the general state of the art which is not considered to be of particular relevance
- *'E' earlier document but published on or after the international filing date
- *'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *'O' document referring to an oral disclosure, use, exhibition or other means
- *'P' document published prior to the international filing date but later than the priority date claimed

- *'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *'A' document member of the same patent family

3

Date of the actual completion of the international search

17 September 1997

Date of mailing of the international search report

01.10.97

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Groenendijk, M

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 97/03036

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 2 - 5
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte	Application No
	PCT/EP 97/03036

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2239178 A	26-06-91	AT 177788 A AU 618270 B BE 1001079 A CA 1328402 A CH 677449 A CS 9104115 A CY 1631 A CY 1632 A DE 3822557 A FI 93308 B FR 2617714 A GB 2208200 A,B GR 1000172 B HK 16492 A HK 16592 A IE 61908 B JP 7048272 A JP 1031728 A JP 2578477 B LU 87268 A NL 8801734 A NO 179359 B PT 87957 B SE 503191 C SE 8802569 A	15-02-97 19-12-91 04-07-89 12-04-94 31-05-91 17-06-92 10-07-92 10-07-92 19-01-89 15-12-94 13-01-89 15-03-89 15-11-91 06-03-92 06-03-92 30-11-94 21-02-95 02-02-89 05-02-97 08-03-89 01-02-89 17-06-96 01-03-95 15-04-96 11-01-89
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